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APPROPRIATE DURATIONS AND MEASURES FOR *CERIODAPHNIA* TOXICITY TESTS

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16. ABSTRACT

The Mount-Norberg test, which employs a measure of the size of three broods over seven days, has been used extensively in toxicity testing. We have applied it to estimating sublethal ecosystem effects of complex effluents in the Raisin River drainage (of Michigan) on the downstream, food-rich communities of Lake Erie. Using an expanded but traditional life-table approach, we observed that the 7-day test provided estimates of natality (M_x) which significantly differed from bioassay results of 14 to 21 days duration. Therefore we conducted an analysis of interactions between duration (times of 0-7, 0-14, 0-21, 0-28 days) and treatment (toxicant levels), and found an appropriate duration of 14 days for food-rich Lake Erie environments. (The controls were run using Lake Erie water; because our conclusions relate to impact of complex effluents upon the lake). In contrast, data from other environments indicate that 5 or 7-day tests are appropriate; an example is provided from a food-poor environment (Lake Superior), where significant results were obtained after 7 days. The population indices natality, survivorship, and intrinsic growth rate (r) are compared for their suitability for use in such tests. Natality was selected as most indicative of toxic effects, especially when delayed effects were involved. The intrinsic growth rate was most affected by early natality and not greatly influenced by broods produced after 7 days.

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INTRODUCTION

The Mount-Norberg three brood toxicity test for Ceriodaphnia is convenient for testing complex mixed effluents on site. At 25°C, the production of three broods is typically completed in seven days [1].

In involving ecosystem characteristics into toxicity testing, it is desirable to simulate temperatures and levels of trophy (food availability) characteristic of the systems receiving the test effluents. Thus we have observed and selected four characteristics of Ceriodaphnia tests which deserve further attention:

a. what is the necessary duration, or the minimum number of broods produced, to obtain significant results when using warm versus cold control waters?

b. what temperature extremes are useful in such modified, ecosystem simulating tests?

c. how can information on the synchronous release of Ceriodaphnia neonates be used to simplify such tests, in the hope of increasing the information obtained at little additional cost.

d. should the chronic endpoint be determined using the number of young/female or the intrinsic growth rate (r)?

This paper presents answers to these questions.

EXPERIMENTAL PROCEDURE

Ceriodaphnia were mass cultured as reported elsewhere [1]. The natural algae and bacteria in control waters from Lakes Erie and Superior were supplemented with a water suspension of activated yeast; 0.05 ml of yeast suspension was added with fresh control water every day. Individual animals were cultured in 15 ml pyrex beakers containing 10 ml of control or test solution; test cultures were started with neonates of C. affinis/dubia less than 24 hr. of age. The species of cladoceran employed was identified by Dorothy Berner of Temple Univ.

Test animals were maintained in cabinets (Percival I-60) with temperature control of $\pm$ 0.5 ^{o}C under a maximum of 7000 lux (700 f.c.) of light.

Life tables were calculated using the methods of Birch [2].

EXPERIMENTAL RESULTS

Duration of Observation

In the relatively food-rich waters of Lake Erie, three broods were produced in control water in 28 days at 18°C (Fig. 1). The same number of broods were produced in experimental containers to which was added 10, 25 and 50% by volume complex mixed effluent from the Raisin River; the characteristics of that effluent are not necessarily pertinent to this discussion. What is vital to see is that differences in the controls and experimental treatments were not significant until 28 days, with the production of three broods. Time and treatment effects were investigated with a 2-way analysis of variance (ANOVA) with replication; the significance of the F value is shown in Table 1.

In the relatively food-poor waters of Lake Superior, three broods were produced in control water in 7 days at 25°C. Again time and treatment effects were investigated with a 2-way ANOVA and shown in Table 1. Two broods produced in 6 days in controls were significantly different in numbers young/female from same measure in experimental treatments involving effluent water from a refinery.

Clearly the concept of a three brood test is valid under different conditions of food and temperature. The time for production of three broods is increased greatly at lowered temperatures, as is the total production of offspring, reduced as will be seen below. Reproductive measures were made over the normal lifetime of the test animals, so that a true measure of net reproductive rate was available from our life tables.

Useful Temperature Extremes

Ceriodaphnia is extremely ubiquitous, occuring in a wide variety of environments. For those who plan to use it to examine the impact of complex effluents upon natural aquatic systems observation of the net reproductive rate R_o at ambient temperature may be more indicative of toxic impact than observation of R_o at 25°C. That is, during decreased ecosystem metabolism at low temperatures, complex effluents may exhibit reduced toxicity.

By raising animals at temperatures lower than the suggested 25°C [1], we have determined that a modified Mount-Norberg toxicity test may be run at temperatures as low as 12°C (Table 2). At 18°C we found 113 young/♀/lifetime of 77 days. At 12°C we found only 13 young/♀/lifetime of 24 days, and at 6°C only 2 young/♀/lifetime of 24 days. Cultures at 18°C were maintained at photoperiods of 16 L/8D, whereas animals grown at 12°C and 6°C were kept in continuous light (24L) to stimulate ovulation.

Synchronous Release of Neonates

Test animals grown at constant temperature (18°C) and started with neonates of similar age (< 24 hours) exhibited synchrony in the release of neonates at approximately 7 day intervals (Figure 1). Young were released synchronously regardless of toxicity level (% complex effluent added to control water). Thus toxicity greatly effects the numbers of young produced per female per time, but not the development time and subsequent release of those neonates (Table 3).

Because of such reproductive synchrony, it should be possible to utilize toxicity tests of very long duration to maximize information on effluents with low concentrations of a slow acting contaminants. While neonates from individual females kept in individual containers might be counted every 7 days (at 18°C), it would still be necessary to feed at least every two days. However, feeding is not as time consuming as counting offspring. Likewise, the renewal of contaminated effluent in cultures would have to be rescheduled. Certainly counting neonates at seven day intervals would provide valuable information on the size of broods later than the third brood, and at little additional cost.

Use of Intrinsic Growth Rates (r)

The measures of natality and survivorship employed in toxicity testing are currently under serious debate. C. Stefan of EPA-Duluth asked us to consider whether the instantaneous growth rate (r) constitutes a better measure of toxic inhibition of natality than the net reproductive rate (R_0). Estimates of r from truncated life tables are plotted (Figure 2) for the same data set shown in Figure 1. While $l_x m_x$ is cyclic, as young were produced synchronously, we observe that r increased for the first 15 days. Thereafter the slope of r was constant. In normal life-table calculations, r is determined at the termination of the lifespan of the adult population. In using truncated life tables we are prone to produce artificially low estimates of r if the asymptote has not been reached. Yet because of the constant slope of r it is possible to estimate r at the normal termination of the lifetable from a value determined about day 11-15, a shorter time than the 28 days required in using natality (m_x). If validated in future studies, this method might be used to abbreviate the necessary period of observation necessary for toxicity testing.

CONCLUSION

The three brood seven day toxicity test using Ceriodaphnia grown at 25°C became a twenty-eight day test at 18°C. Varying temperature will become an important part of this test as it is applied to complex ecosystems. Generally Ceriodaphnia can be grown at temperatures of 12 to 25°C; too few neonates were produced at 6°C for useful test results. The synchronous release of neonates by Ceriodaphnia females may be used in determining when to sample offspring and reduce the cost of testing complex effluents. The intrinsic growth rate of Ceriodaphnia may be used early in a test to predict the growth rate at the termination of a normal adult female's lifespan.

FIGURES

Figure 1. Variation in the product of survivorship (l_x) and natality (m_x) for Ceriodaphnia raised over 40 days in control and experimental (10% and 50% effluent) cultures.

Figure 2. Variation in the intrinsic growth rate (r) of Ceriodaphnia calculated from truncated life tables from day 1 through 40 for control and experimental cultures.

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1. Mount, D.I. and T.J. Norberg. A seven-day life-cycle Cladoceran toxicity test. M.S.

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TABLE 1. Time of observation needed for significant treatment effects.

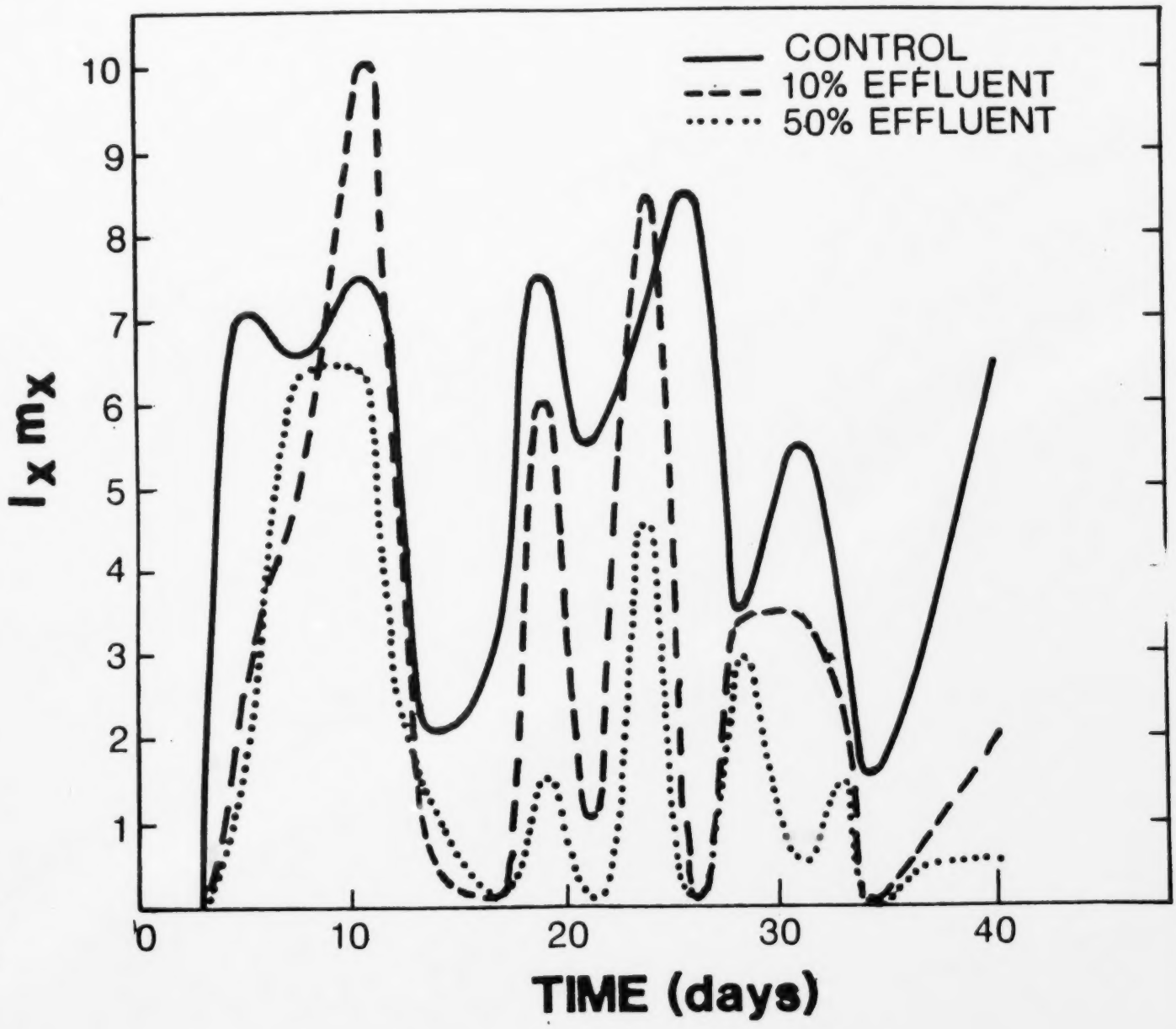
	Environments							
	Lake Erie (October 1983)				Lake Superior (September 1982)			
Observation Period of Test (days)	7	14	21	28	4	5	6	7
Time	NS	NS	NS	$p<.10$	NS	NS	$p<.005$	$p<.005$
Treatment level	NS	NS	NS	$p<.10$	NS	NS	$p<.005$	$p<.005$
Interaction	NS	NS	NS	NS	NS	NS	$p<.005$	$p<.005$

TABLE 2. Decreasing net reproductive rate (R_o) with temperature (^oC), (for *Ceriodaphnia* grown in L. Erie water supplemented with yeast).

Temperature	R_o	(Photoperiod)
18°C	113 young/♀	16L/8D
12	13	24L/0D
6	2	24L/0D

TABLE 3. Decrease in net reproductive rate (R_o) of Ceriodaphnia with increasing amounts of complex effluent from Raisin River, at 18°C. (October 1983).

Effluent from Site#	Control	10% effluent	25% effluent	50% effluent
8	54	32	8	15 ind ind^{-1}
10	54	22	46	49


CONTROL
10% EFFLUENT
50% EFFLUENT
$l_x m_x$
10
9
8
7
6
5
4
3
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1
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30
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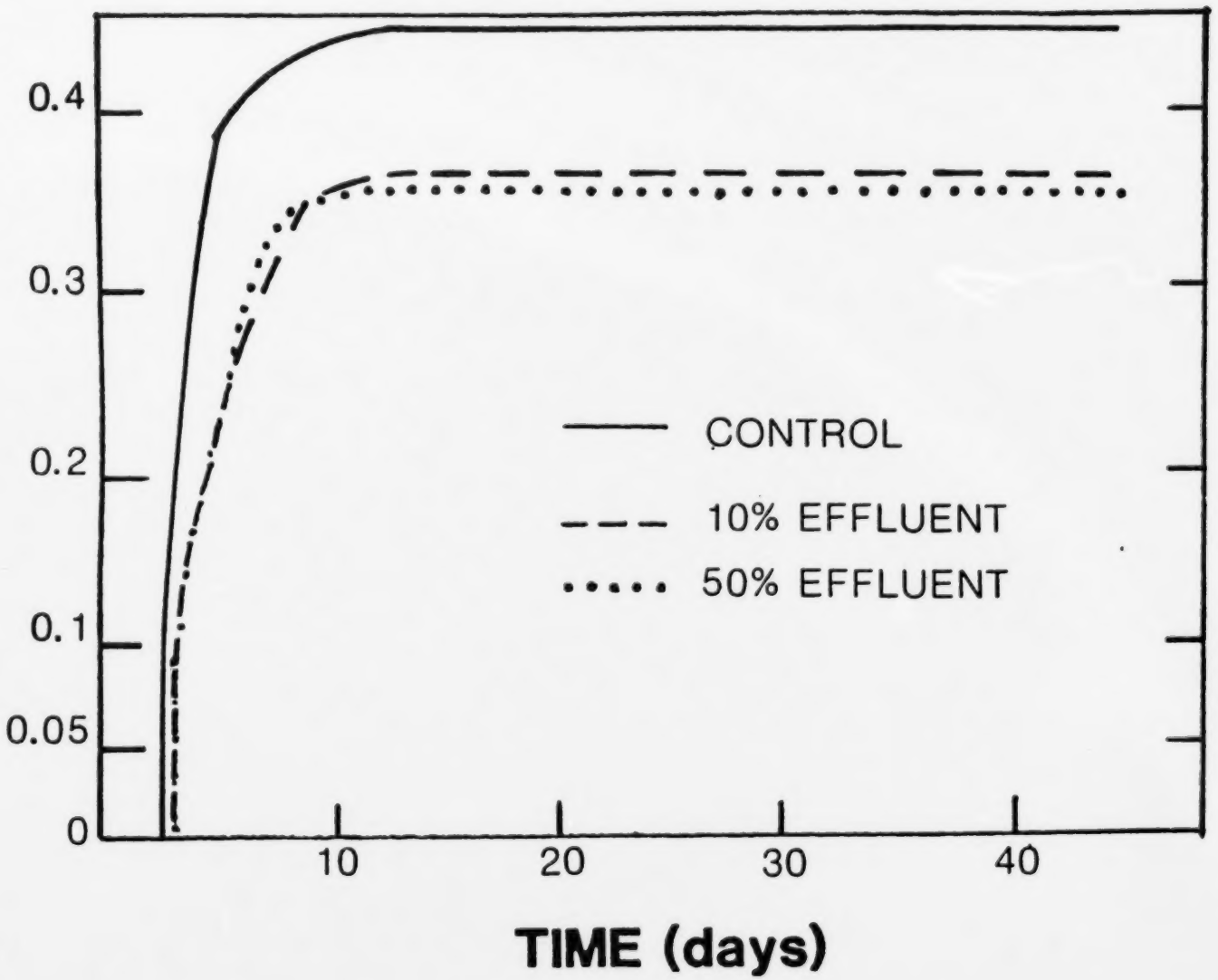
TIME (days)


0.4
0.3
0.2
0.1
0.05
0
10
20
30
40
CONTROL
10% EFFLUENT
50% EFFLUENT
TIME (days)